Cautions in Using Rapid Tests for Detecting Influenza A Viruses

This communication is intended to provide information about rapid influenza tests[1] currently available in the US market, their uses and limitations for detecting Influenza A viruses, and their performance implications. It is also intended to reiterate guidance provided by CDC on the recommended use of these tests.

Background: Concern with the spread of avian influenza A (H5N1) virus among wild birds and poultry along with sporadic, often fatal human infections has heightened awareness for the potential of an emerging influenza strain that could be transmitted readily person-to-person. FDA considers it a high priority to support the Department of Health and Human Services initiative to prepare for human infections with emerging strains of influenza A virus with pandemic potential.[2]

A variety of laboratory tests can be used to detect influenza A viruses directly in human clinical specimens. These include viral culture, polymerase chain reaction (PCR),[3] immunofluorescence DFA testing, and enzyme immunoassays for influenza A virus antigens, along with the rapid influenza tests. An understanding of the current level of influenza activity (i.e., prevalence) and test limitations is critical for appropriately using rapid testing to guide treatment decisions for individual patients and for applying infection control measures to prevent transmission in health-care and other settings. In addition to diagnosis of individual patients, testing is needed to maintain vigilance for newly emerging influenza A subtypes and for monitoring influenza activity. Culture and methods other than rapid testing are essential for detecting influenza infection missed by rapid testing, for confirming influenza infection particularly when prevalence is low, for monitoring influenza A subtypes and strains circulating in the U.S., for annual vaccine strain selection, and for monitoring potential emergence of resistance to antiviral drugs.

FDA-cleared rapid influenza tests: FDA has cleared several rapid tests with various formats (e.g., dipstick-type, lateral flow immunochromatographic cassettes) for use in clinical laboratories, including CLIA-waived settings. These tests may detect:

- either influenza A or B virus without identifying the type (or types),
- influenza A virus only, or
- both influenza A and B virus and identifying the type (or types).

The last two tests detect a conserved influenza A virus nucleoprotein that differentiates influenza A from influenza B, using specific antibodies. At the present time, none of the FDA-cleared rapid influenza A tests can differentiate influenza A virus subtypes or discriminate between those subtypes that commonly infect humans (e.g., H3N2 and H1N1) and those that typically infect birds.[2]

FDA clears these tests for distribution in the U.S. based on data from studies using both clinical specimens from patients in various clinical settings, and from analytical-type studies with cultured virus. Studies with cultured virus are important to assure reactivity of the anti-nucleoprotein monoclonal antibodies used in these tests with a variety of influenza A virus subtypes. Table 1 shows analytical limits of detection across different strains for several influenza A subtypes, using two different laboratory methods to quantify cultured virus.

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[1] For purposes of this document, rapid influenza tests are those that can detect influenza virus antigens or viral enzyme activity within 30 minutes (see http://www.cdc.gov/flu/professionals/labdiagnosis.htm) and does not include traditional test methods such as immunofluorescence DFA antibody staining or PCR testing that can be done in 2-4 hours.

[2] Information sources such as http://www.cdc.gov/flu/avian/gen-info/flu-viruses.htm describe influenza virus types, subtypes and strains.

[3] Influenza PCR tests are available in some laboratories. These are either investigational or laboratory-developed assays. At the present time they have not been cleared by FDA for diagnostic use.

Table 1. Ranges of Analytical Limits of Detection: Data from two tests cleared during the past few years.

Influenza A Subtype	Limit of Detection	
	$CEID_{50}/mL$[a]	$TCID_{50}/mL$[b]
A/H1	2.8×10^2 - 1.8×10^4 (6 strains)	1.0×10^3 - 6.0×10^5 (3 strains)
A/H2	ND	1.2×10^3 (1 strain)
A/H3	1.0×10^3 - 2.8×10^4 (3 strains)	9.0×10^2 - 2.3×10^6 (5 strains)
A/H5	ND	1.0×10^3 (1 strain)

a = Chick embryo infectious dose 50% ND= Not Done
b = Tissue culture infectious dose 50%

The studies with cultured virus, however, cannot be used to infer efficient detection of specific influenza A subtypes from respiratory samples. Additionally, these rapid tests are evaluated in clinical studies using various specimen types collected from patients with influenza-like illness during an influenza season. These studies compare the rapid test results to traditional detection methods (i.e., culture and/or immunofluorescent assays). Subtyping of influenza A positive cases is not done.

Optimum specimens for influenza virus cultures are nasopharyngeal aspirates obtained within three days of onset of symptoms.[4] Rapid influenza tests have also been evaluated with other specimen types such as nasal and throat swabs. It is well-recognized that testing done with children will appear more sensitive because children shed virus more abundantly and longer than adults.[5] Predictably, with data submitted to FDA, sensitivity of these tests is generally higher in pediatric populations, and when using nasopharyngeal aspirate or nasal wash specimens (see Table 2). Specificity also may vary by age and specimen type.

Table 2. 95% Confidence Intervals: Data from two tests cleared during the past few years.

Specimen Type	Influenza Virus Type Detected	Population[a]	Sensitivity (95% CI)[c]	% Specificity (95% CI)[c]
Throat swab	Influenza A	Pediatric[b]	65 to 90	81 to 91
		Adult	24 to 91	69 to 94
Throat swab	Both Influenza A & B	Not specified	59 to 82	81 to 93
Nasopharyngeal wash/aspirate	Influenza A	Pediatric[b]	82 to 95	98 to 100
		Adult	53 to 87	90 to 100
Nasal wash	Influenza A	Pediatric[b]	36 to 88	92 to 99
		Adult	9 to 99	59 to 100
Nasal wash and aspirate	Influenza A	Not specified	65 to 84	95 to 99
Nasal swab	Both Influenza A & B	Not specified	65 to 87	87 to 97

[a] From the U.S., Australia, or New Zealand during seasons where A/H3 and A/H1 were predominant circulating influenza A viruses (derived from WHO Flunet, http://gamapserver.who.int/GlobalAtlas/home.asp)
[b] Age range not specified; majority are <10 years [c] 95% Confidence Interval

These sensitivity and specificity ranges will be expected to be similar in clinical laboratory practice with the predominant circulating influenza A viruses. If a new subtype emerges, additional studies

[4]WHO, Recommended laboratory tests to identify avian influenza A virus in specimens from humans, June 2005, http://www.who.int/csr/disease/avian_influenza/guidelines/avian_labtests2.pdf
[5] WHO recommendations on the use of rapid testing for influenza diagnosis, July 2005, available at http://www.who.int/csr/disease/avian_influenza/guidelines/RapidTestInfluenza_web.pdf

would be needed to verify similar clinical performance. At this time, preliminary information from rapid antigen testing in Asia suggests poor sensitivity compared with culture-positive human influenza A (H5N1) cases.[6] Furthermore, the best clinical specimen to use for detecting H5N1 infections is not known.

Performance Implications: Rapid tests have been shown to have moderate sensitivities when compared to traditional detection methods for influenza A and B virus strains circulating during the influenza season (during which each test was evaluated). Although rapid influenza tests cleared for use in the U.S. generally demonstrate a sensitivity of >60%, false negatives are likely, and may vary by age and type of specimen. While specificity of cleared rapid tests is generally high (>90-95%), false positive test results occur and again may vary by age and specimen type.

Importantly too, positive and negative predictive values of these tests are highly dependent on prevalence, or current level of influenza activity.[7] During peak influenza activity in a season, positive predictive values are higher, with false positives less likely; and negative predictive values are lower, with false negatives more likely. Conversely, during low influenza activity (e.g., off-season or beginning of a season), negative predictive values are higher and positive predictive values lower, with false positive test results more likely.

Finally, although several of the rapid influenza tests have been demonstrated to analytically detect different subtypes, the performance of these tests has not been established for the clinical detection of any particular influenza A subtype, except for A/H3N2 and A/H1N1. At this time, it is unknown whether similar sensitivity and specificity would be experienced when a new subtype emerges as a predominant circulating influenza A type.

Guidelines and Recommendations: Respiratory illness caused by influenza is difficult to distinguish from illness caused by other respiratory pathogens on the basis of symptoms alone, [8] and rapid testing may be helpful for guiding antiviral treatment decisions and control activities.

CDC and WHO outline recommended strategies for influenza diagnostic testing for inpatients and outpatients.[9] Rapid tests may be useful in these strategies by providing preliminary tools for guiding treatment and patient management in a clinically relevant time frame (less than 30 minutes). Also, during a respiratory illness outbreak in an institution or particular community setting, rapid influenza tests can be helpful in determining if influenza is the cause of the outbreak.

As recommended by CDC,[10] when avian influenza viral infection is suspected based on exposure risk, testing of a nasopharyngeal swab or aspirate should be requested through state and local health departments. Specimens should be collected following infection control precautions for influenza A (H5N1) and testing done using standard BSL 2 work practices in a Class II biological safety cabinet. [11]

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[6] Writing Committee of the World Health Organization Consultation on Human Influenza A/H5. Avian Influenza A (H5N1) Infection in Humans. NEnglJMed 2005, 353(13):1374-85.

[7] WHO recommendations on the use of rapid testing for influenza diagnosis, July 2005, available at http://www.who.int/csr/disease/avian_influenza/guidelines/RapidTestInfluenza_web.pdf

[8] http://www.cdc.gov/flu/professionals/diagnosis/

[9] Interim Guidance for Influenza Diagnostic Testing During the 2004-05 Influenza Season, November 2004, http://www.cdc.gov/flu/professionals/diagnosis/0405testingguide.htm

[10] CDC Health Update on SARS and Avian Influenza A (H5N1), Health Alert Network, June 2004. available at http://www.phppo.cdc.gov/HAN/ArchiveSys/ViewMsgV.asp?AlertNum=00204

[11] Update on Influenza A(H5N1) and SARS: Interim Recommendations for Enhanced U.S. Surveillance, Testing, and Infection Control, February 2004. htpp://www.cdc.gov/flu/avian/professional/han020302.htm

Limitations: When interpreting results from any rapid influenza test, laboratorians and clinicians must use clinical experience, further laboratory testing, surveillance information about circulating influenza strains and the current level of influenza activity, along with an understanding of the limitations of these rapid tests. Some of these limitations that apply for seasonal human influenza and most likely for an outbreak with a new influenza A virus are:

- When influenza activity is low, positive results should be confirmed by immunofluorescence DFA testing, viral culture, or RT-PCR as false positive test results are more likely. Additionally, a positive test does not rule out at any time, the possibility of co-infections with other pathogens.[9]
- During peak activity when negative predictive values are lowest, false negatives are more likely. Particularly at the beginning of the season or outbreak, negative results may not be relied on to guide management or treatment decisions. Confirmatory testing using immunofluorescence, viral culture, or PCR must always be considered because a negative test may not rule out influenza viral infection.
- None of these tests provides any information about influenza A subtypes, and none can differentiate influenza A strains that infect humans from those strains that typically infect birds or other animals (e.g., H5, H7, H9). Additional testing is needed to obtain information on influenza A subtypes.[12]
- The tests may have lower sensitivity for adults than for children because children tend to shed virus more abundantly and for longer periods of time.

Additionally, FDA cautions clinicians and laboratorians assessing the qualities of individual tests to carefully consider published literature, with the following caveats:

- Test sensitivities and specificities cannot necessarily be compared across package inserts as these studies were done with different patient groups, with different levels of influenza activity, at different times post onset of symptoms, with different specimen types, and under different laboratory conditions.
- The performance of any rapid test using frozen samples may likely show greater sensitivity than with freshly collected samples.[13]
- Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false negative tests. Training in specimen collection is highly recommended because of the importance of specimen quality.[14]
- Predictive values of individual tests will depend on the level of influenza activity in the community, the types of circulating viruses at the time, the age of patients, and the adequacy of specimen collection.

[12] Influenza type A viruses can infect people, birds, pigs, horses, seals, whales, and other animals, but wild birds are the natural hosts for these viruses (http://www.cdc.gov/flu/avian/gen-info/avian-influenza.htm)
[13] Quach et al. 2002 Clin Diagn Lab Imm; 9:925-926
[14] WHO recommendations on the use of rapid testing for influenza diagnosis